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Haemato-biochemical Alteration in Bacteria Associated Respiratory Tract Infection in Dog

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ABSTRACT

The research was undertaken during October, 2022 to June, 2023 to study the changes in haemato-biochemical values of the dogs suffered from respiratory tract infection due to bacteria. Blood samples were collected from healthy control group (Group A) and each dog of different treatment groups (Group B, Group C and Group D) on the 0th day (pre-treatment) and the 10th day (post-treatment) in K_3-EDTA vacutainer tubes as well as clot activator-coated vacutainer tubes for haemato-biochemical estimation. In the result of haemato-biochemical study the highly significant difference ($^{**}p<0.01$) was found in total leucocyte count, absolute neutrophil count and C-reactive protein. Statistically no significant difference ($p>0.05$) was observed in total erythrocyte count, haemoglobin, absolute eosinophil count, absolute lymphocyte count and albumin. In case of total protein a highly significant difference ($^{**}p<0.01$) was observed between Group A and Group B on day 0 and on day 10. A highly significant difference was also observed between ($^{**}p<0.01$) Group B and Group C; Group B and Group D on day 0 and day 10. On the other hand no significant difference ($p>0.05$) was observed within treatment groups. In case of globulin, statistically no significant difference ($p>0.05$) was obtained between the Group A and all the treatment groups on day 0 and day 10. No significant difference ($p>0.05$) was observed within treatment groups. However, a significant difference ($^{**}p<0.01$) was found between Group B and Group C; Group B and Group D on 0th day and on the 10th day.

KEYWORDS: Blood, c-reactive protein, total leucocyte count

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1. INTRODUCTION

Dog (*Canis familiaris*) is a faithful pet animal and provide emotional support to the owner. In the year 2012 dog Population was around 10.2 million in India (Bradley and King, 2012). First domesticated animal is dog and have interaction with human beings (Freedman and Wayne, 2017). In a research study by Pescini et al., 2019 explained that dogs as well as human both release oxytocin and they inferred that oxytocin is the main reason for creating a strong social bond between human being and pet animal. Anatomically respiratory tract is broadly classified into upper respiratory tract and lower respiratory tract. The upper respiratory tract includes nose, nasal sinus, larynx and the lower respiratory tract contains trachea, bronchi, bronchiole, alveoli and lungs. According to Beeler-Marfisi et al., 2020 respiratory system helps in gaseous exchange, maintaining acid-base balance, protection of airways by warming and humidifying inhaled air, olfaction and filtering out the dust by the muco-ciliary movement of epithelium. Apart from muco-ciliary defence mechanisms mucus also has some protective factors such as lactoferrin which is bacteriostatic where as lysozyme and $alpha_1$ antitrypsin have bacteriolytic properties. T-lymphocytes release lymphokines which give cell mediated immunity to lungs. Bronchus Associated Lymphoid Tissue (BALT) and alveolar macrophages also play significant roles in pulmonary defensive mechanisms. (Chakrabarti, 2014). Various Infectious diseases *viz.* canine distemper, infectious canine hepatitis, parvo viral enteritis, rabies, leptospirosis, aspergillosis etc. and non-infectious diseases namely congestive heart failure, cardiomyopathy, degenerative joint diseases, hip dysplasia, diabetes mellitus, renal disorder etc. occur in the dog due to breed predisposition, age and hereditary origin. Rabies, leptospirosis, scabies, dermatophytosis, yersinosis, cryptosporidiosis, MRSA etc. are a few zoonotic diseases which can be transmitted from dogs to humans and cause serious life-threatening illness in personnel with specific medical conditions like immunodeficiency, chronic illness, pregnancy etc. Since dogs are living with human in close contact, they are quite frequently exhibiting respiratory tract infections involving both upper and lower respiratory tract. Different researchers *viz.* Qekwana et al., 2020; Moon et al., 2022; Moyaert et al., 2019 and Yadav et al., 2020 found that in RTI of dogs, various kind of bacteria are involved *viz. Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli*, *Pasteurella* spp., *Klebsiella* spp., *Pseudomonas* spp., *Bordetella bronchiseptica* etc. During the infection, patient exhibit different clinical manifestation like dyspnoea, fever, coughing, sneezing, nasal discharge, lethargy and weight loss etc (Sanchez-Viscaino et al., 2016; Ellis et al., 2016; Arsevska et al., 2018 and Kashyap et al., 2019). In many instances few of the clinical manifestation turns to chronic and become resistant to the commonly used antibiotics. Out of various pathogens bacteria is one of the most common involvements for the onset of respiratory tract infection. Methicillin-resistant *Staphylococcus aureus* is normally present on the skin of healthy dogs and human, but these bacteria can opportunistically cause skin infection, respiratory tract as well as urinary tract infection. In immune-compromised animal normally inhabiting bacteria opportunistically cause respiratory tract infection (Jose and Brown, 2016 and Debnath et al., 2022). Haemato-biochemical changes *viz.* absolute neutrophil count, total leucocyte count, inflammatory biomarkers like C-reactive protein etc. give some clue for assessment of bacteria associated respiratory tract infection in dogs (Viitanen et al., 2014; Kaur et al., 2013; Prat and Lakoma, 2016; Hong et al., 2021; and Menard et al., 2022). In North Eastern Region of India, investigation on bacteria associated respiratory tract infection in canine has not been studied so far. Considering the importance, present study was undertaken to determine the changes in haemato-biochemical parameters in canine respiratory tract infection.

2. MATERIALS AND METHODS

2.1. *Place of work and source of animal*

Dogs presented to the Veterinary Clinical Complex (VCC), College of Veterinary Science, Assam Agricultural University, Khanapara from different parts of Assam and neighbouring states of the North-Eastern Region during the period from October, 2022 to June, 2023 were considered for the study.

2.2. *Physical examination of suspected dogs*

Dogs presented in the Veterinary Clinical Complex (VCC) with a history of coughing, sneezing, presence of nasal discharge, respiratory distress including clinical symptoms *viz.* pattern of nasal discharge, types of coughing, rectal temperature, sneezing, auscultation of lungs etc. were recorded during clinical examination.

2.3. *Haemato-biochemical examination*

Ten blood samples were collected from healthy control group namely Group A and each dog of different treatment groups (Group B, Group C, Group D) on the 0^{th} day (pre-treatment) and the 10^{th} day (post-treatment) of registration of the dog to Veterinary Clinical Complex (VCC) for estimation of haemato-biochemical parameters. Approximately four millilitres of whole blood were collected from the cephalic or saphenous vein of the dog. A fraction of whole blood was collected in K_3-EDTA vacutainer tubes for estimation of haematological parameters using automated haematology cell counter (Model MS4e, Melet Schloesing, France). The remaining fraction of blood was transferred to clot activator-coated vacutainer tubes for evaluation of biochemical parameters using fully automatized machine IDEXX Catalyst One and C-reactive protein (CRP) was estimated by ELISA kit, marketed by Bioassay Technology Laboratory, Shanghai Korain Biotech Co., Ltd., 228 Ningguo Rd, Yangpu Dist, Shanghai, China.

2.4. Bacteriological examination

Nasal swab samples were collected by using sterile swabs aseptically for isolation and identification of the bacteria up to genus level. Further identification of bacterial isolates was done by Gram's staining and series of biochemical test *viz.* catalase test, indole test, methyl red test, Voges Proskaures test and citrate utilization test.

3. RESULTS AND DISCUSSION

3.1. Total leucocyte count (TLC)

The mean TLC (m $mm^{-3/2}$) is presented in the Table 1. In statistical analysis, on day 0, a significant difference ($^{**}p<0.01$) was observed between Group A (healthy control) and three treatment groups *viz.* Group B, Group C and Group D. On day 0 (pre-treatment) and on day 10 (post-treatment) between the three treatment groups no significant difference ($p>0.05$) was observed. However, a significant difference ($^{**}p<0.01$) was observed within the three treatment groups. The mean values of TLC (m $mm^{-3/2}$) towards the end of treatment, were found within the normal range which was in accordance to the findings of Kaneko et al. (2008). Similar findings were observed by Kose et al. (2021) which indicated that the groups were responded to the therapeutic management. Various researchers also reported similar findings who observed higher levels of TLC in the infected animals than the healthy control (Elgalfy et al., 2022, Menard et al., 2022 and Hong et al., 2021). The high level of TLC might be due to inflammation and the involvement of bacteria associated infection (Priestnall and Erles, 2011).

3.2. Total erythrocyte count (TEC)

The mean TEC (M $mm^{-3/2}$) is presented in the Table 1. Statistically no significant difference ($p>0.05$) was observed between Group A and three treatment groups on day 0 and day 10 and similarly no significant difference ($p>0.05$) was observed within the treatment groups. In the present study, a non-significant decrease of TEC in different treatment groups on day 0 was observed. However, the mean values of TEC (M $mm^{-3/2}$) were towards the increasing fashion in all the treatment groups after rendering treatment. In the present study, a non-significant decrease of TEC in different treatment groups on day 0 was observed. However, the mean values of TEC (M $mm^{-3/2}$) were towards the increasing fashion in all the treatment groups after rendering treatment. Similar findings were observed by Köse et al. (2021). However, Hong et al. (2021) reported a significantly

Table 1: (Mean±SE) of total leucocyte count (m $mm^{-3/2}$),total erythrocyte count (M $mm^{-3/2}$), haemoglobin (g dl^{-1}), absolute neutrophil count (m $mm^{-3/2}$), absolute eosinophil count (m $mm^{-3/2}$), absolute lymphocyte count (m $mm^{-3/2}$), total protein (g dl^{-1}), albumin (g dl^{-1}), globulin (g dl^{-1}) and C-reactive protein (mg dl^{-1}) in pre and post-treatment days

Parameters	Groups	Pre-treatment (Day 0)	Post-treatment (Day 10)
Total leucocyte count (m $mm^{-3/2}$)	Group A	$10.17±0.92^a$	
	Group B	$19.26±2.69^b$	$14.03±2.93^a$
	Group C	$22.96±3.30^b$	$14.65±0.79^a$
	Group D	$20.87±2.87^b$	$11.18±0.66^a$
Total erythrocyte count (M $mm^{-3/2}$)	Group A	7.52±0.65	
	Group B	6.45±0.60	6.56±0.58
	Group C	6.45±0.26	7.24±0.51
	Group D	6.08±0.59	6.82±0.47
Haemoglobin (g dl^{-1})	Group A	15.28±0.68	
	Group B	12.45±1.17	12.81±0.86
	Group C	13.27±0.35	14.51±0.90
	Group D	11.25±0.98	12.13±0.69
Absolute neutrophil count (m $mm^{-3/2}$)	Group A	$7.29±0.71^a$	
	Group B	$14.58±2.26^b$	$9.51±2.01^c$
	Group C	$17.38±2.32^d$	$11.02±0.92^e$
	Group D	$14.13±2.19^f$	$7.15±0.63^a$
Absolute eosinophil count (m $mm^{-3/2}$)	Group A	0.20±0.02	
	Group B	0.38±0.06	0.31±0.05
	Group C	0.50±0.07	0.32±0.03
	Group D	0.40±0.06	0.43±0.23
Absolute lymphocyte count (m $mm^{-3/2}$)	Group A	2.38±0.24	
	Group B	3.75±0.47	3.69±0.80
	Group C	4.56±0.98	3.06±0.52
	Group D	5.75±1.14	3.17±0.24
Total protein (g dl^{-1})	Group A	$6.63±0.12^a$	
	Group B	$7.27±0.29^b$	$7.17±0.32^b$
	Group C	$6.24±0.17^a$	$6.32±0.21^a$
	Group D	$6.35±0.37^a$	$6.39±0.11^a$
Albumin (g dl^{-1})	Group A	2.62±0.18	
	Group B	2.72±0.13	2.69±0.09
	Group C	2.73±0.13	3.09±0.18
	Group D	2.74±0.12	2.87±0.09
Globulin (g dl^{-1})	Group A	$4.02±0.21^{ab}$	
	Group B	$4.58±0.31^a$	$4.53±0.33^a$

Table 1: Continue...

Parameters	Groups	Pre-treatment (Day 0)	Post-treatment (Day 10)
	Group C	3.61 ± 0.22^{b}	3.57 ± 0.24^{b}
	Group D	3.60 ± 0.13^{b}	3.51 ± 0.08^{b}
C-reactive protein (mg dl^{-1})	Group A	1.68 ± 0.08^{a}	
	Group B	3.60 ± 0.48^{b}	1.73 ± 0.09^{a}
	Group C	2.02 ± 0.23^{a}	1.10 ± 0.16^{a}
	Group D	2.55 ± 0.32^{a}	1.66 ± 0.43^{a}

NB: Means with different superscripts differ significantly

low level of TEC in bacterial pneumonia affected dogs. In the present study, comparatively low level of TEC in the infected animals than the healthy control group due to concurrent haemo-protozoal infection of a few suspected cases.

3.3. Haemoglobin (Hb)

The mean haemoglobin (g dl^{-1}) value is presented in the Table 1. Statistically, no significant difference ($p>0.05$) was observed between Group A and three treatment groups on day 0 and day 10. No significant difference ($p>0.05$) was also observed within the treatment groups. In the current study, a non-significant decrease of haemoglobin in three treatment groups on day 0. However, the mean values of Haemoglobin (g dl^{-1}) were towards the increasing fashion in all the treatment groups after rendering treatment. Similar findings were observed by Kose et al. (2021) and Ayodhya et al. (2013) who found a non-significant low concentration of haemoglobin in dogs suffering from bacteria-associated respiratory tract infection. However, Elgalfy et al. (2022) and Hong et al. (2021) reported a significantly low level of haemoglobin in bacteria-associated pneumonia in affected dogs. In the current study, a comparatively low level of haemoglobin than healthy control in the infected dogs might be due to concurrent haemo-protozoal infection.

3.4. Absolute neutrophil count

The mean absolute neutrophil count (m $mm^{-3/2}$) is presented in the Table 1. On statistical analysis, a significant difference ($p<0.01$) was observed within all the treatment groups on day 0 and day 10. Significant difference ($^{**}p<0.01$) was observed between the Group A and in all the treatment groups on day 0. Significant difference ($^{**}p<0.01$) was also observed between Group A and in two treatment groups *i.e.* Group B and Group C except Group D on day 10. The present study revealed a significant decrease in absolute neutrophil count was observed in all the treatment groups on day 10. These findings were in accordance with Köse et al. (2021) which showed that the groups were responded to the treatment. In this study, there was a significant increase of mean absolute neutrophil count, observed in Group B, Group C and Group D than that of the healthy control group on day 0. These observations were in accordance with Elgalfy et al. (2022), Menard et al. (2022), Hong et al. (2021), Priestnall and Erles (2011) and Amrute et al. (2009) who found increased neutrophil count in the affected dogs. The high level of neutrophil count on Day 0 in the present study might be due to the migration of neutrophil directly to the site of infection as neutrophil is considered essential mediators for pulmonary immunity, accumulates in the site to clear and control the infection (Pechous, 2017).

3.5. Absolute eosinophil count

The mean absolute eosinophil count (m $mm^{-3/2}$) is presented in the Table 1. Statistically no significant difference ($p>0.05$) was observed between Group A and three treatment groups on day 0 and day 10. Similarly, no significant difference ($p>0.05$) was observed within the treatment groups. In case of absolute eosinophil count, no significant difference ($p>0.05$) was observed within the treatment groups. Similar findings were observed by Menard et al. (2022) and Hong et al. (2021).

3.6. Absolute lymphocyte count

The mean absolute lymphocyte count (m $mm^{-3/2}$) is presented in the Table 1. Statistically no significant difference ($p>0.05$) was observed between Group A and three treatment groups on day 0 and day 10. Similarly, no significant difference ($p>0.05$) was observed within the treatment groups. A non-significant high absolute count of lymphocytes in all the treatment groups on day 0. However, the absolute lymphocyte count was towards the decreasing manner in the three treatment groups after rendering treatment. The present study revealed a non-significant high absolute count of lymphocytes in all the treatment groups on day 0. However, the absolute lymphocyte count was towards the decreasing manner in the three treatment groups after rendering treatment. These findings were in accordance with Goggs et al. (2022) and Ayodhya et al. (2013) who found no significant difference in lymphocyte count in the affected animals and Viitanen et al. (2017) found lymphopenia in the bacteria-associated respiratory tract infected dogs. However, on the contrary Hong et al. (2021) found a significant increase of lymphocyte count in the affected dogs. A mild high level of lymphocyte count on Day 0 might be due to infection.

3.7. Total protein (TP)

The mean total protein (g dl^{-1}) is presented in the Table 1. On statistical analysis significant difference ($^{**}p<0.01$) was observed between Group A and Group B on day 0 and on day 10. A significant difference was also observed between ($^{**}p<0.01$) Group B and Group C; Group B and Group D on day 0 and day 10. No significant difference ($p>0.05$) was observed within treatment groups. Elgalfy et al. (2022) and Hong et al. (2021) observed higher levels of total protein in the bacteria-associated respiratory tract infection in dogs which were similar to current findings. The slight increase of total protein in the affected animal might be due to an increase in the globulin portion of total protein during the infection period.

3.8. Albumin (ALB)

The mean ALB (g dl^{-1}) level is presented in the Table 1. Statistically, no significant difference ($p>0.05$) was observed between Group A and three treatment groups on day 0 and day 10. No significant difference ($p>0.05$) was also observed within the treatment groups. In case of albumin no significant difference ($p>0.05$) was observed within the treatment groups. These findings were supported by Goggs et al. (2022) who found albumin in the normal range in pneumonia affected dogs. Amrute et al. (2009) and Hong et al. (2021) found low level of albumin in the infected dogs. In the current study albumin is slightly reduced in the infected individuals which might be due to acute inflammation (Tothova et al., 2016).

3.9. Globulin (GLB)

The mean GLB (g dl^{-1}) is presented in the Table 1. Statistically, no significant difference ($p>0.05$) was obtained between the Group A and all the treatment groups on day 0 and day 10. No significant difference ($p>0.05$) was obtained within treatment groups. However, a significant difference ($^{**}p<0.01$) was found between Group B and Group C; Group B and Group D on 0^{th} day and on the 10^{th} day. In the present study, there was a slight increase in the level of globulin in Group B, Group C and Group D on day 0. However, the level of globulin was towards the decreasing fashion in Group B, Group C and Group D after rendering treatment. In the present study, there was a slight increase in the level of globulin in Group B, Group C and Group D on day 0. However, the level of globulin was towards the decreasing fashion in Group B, Group C and Group D after rendering treatment. Elgalfy et al. (2022) and Hong et al. (2021) found increased globulin concentration in the bacteria associated respiratory tract infections in dogs. In the present study globulin level is slightly high might be due to inflammatory conditions in the body due to respiratory tract infection (Tothova et al., 2016).

3.10. C-reactive protein (CRP)

The mean C-reactive protein (mg dl^{-1}) is presented in the Table 1. A statistically significant difference ($^{**}p<0.01$) was found within Group B before and after treatment. Precisely, a significant difference ($^{**}p<0.01$) was found between only Group A and Group B on day 0. Similarly, significant difference ($^{**}p<0.01$) was observed between Group B and Group C; Group B and Group D on day 0. However, no significant difference ($p>0.05$) was found between the three treatment groups on day 10. In the present study, there was a higher value of C-reactive protein in different treatment groups on day 0. However, the level of C-reactive protein was towards the decreasing manner in different groups after rendering treatment which indicated that all the groups responded to the treatment in terms of the status of CRP. Similar findings were obtained by Nakamura et al. (2008) who found the level of CRP was>1 mg dl^{-1} (75.00%) and >10 mg dl^{-1} (6.00%) in bronchopneumonia affected dogs. Viitanen et al. (2017) found there was a gradual decrease of CRP following antimicrobial treatment for the bacterial pneumonia in dogs. The high serum CRP level may be due to inflammation and damage of tissue. CRP mainly binds with the cell wall residue of microbes containing phosphoryl choline which activates the complement system and further stimulate to produce more CRP (Stewart, 2012).

4. CONCLUSION

In the study of haemato-biochemical alterations in the bacteria associated respiratory tract infection in dog, the highly significant difference was found in total leucocyte count, absolute neutrophil count and C-reactive protein. Total leucocyte count, absolute neutrophil count and C-reactive protein had a highly significance for diagnosing bacteria associated respiratory tract infection in dog.

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